

SUPPLEMENTARY MATERIALS

MATERIALS AND METHODS

The human biological samples used in the following experimentation were collected with the expressed consent, free and informed, to the collection and use, of the person from whom the material was taken, according to current legislation.

Plasma sample preparation

Each plasma sample was treated as follows: 5 μL of CH_3CN were added to 50 μL of plasma and vortexed for 1 minute. The procedure was repeated 10 times. Then the sample was centrifuged at 1,500 g for 10 minutes and two 100 μL aliquots of supernatant were treated under normal and denaturation conditions:

- ***Normal conditions***

The aliquot was dried by nitrogen treatment for 1h and, once fully dried, it was re-suspended in 1,000 μL NH_4HCO_3 50 mM buffer. Filtering by using 0.22 μm bacteriological membrane was applied.

No denaturation process was applied. Desalting was obtained using zip-tip cartridge (Millipore, Italy). After filtration, the sample was eluted in 200 μL of CH_3CN (80%), dried by nitrogen treatment for 30 minutes, and re-suspended in 200 μL NH_4HCO_3 50 mM pH 7.8 buffer solution to allow re-folding. Sorbic acid (solution 1%) was added as antibacterial agent.

- ***Denaturation conditions***

The aliquot was dried by nitrogen treatment for 1h and, once fully dried, it was re-suspended in 1,000 μL NH_4HCO_3 50 mM buffer. Filtering by using 0.22 μm bacteriological membrane was applied.

After filtration, the sample was dried by nitrogen treatment for 30 minutes, re-suspended in 200 μL NH_4HCO_3 50 mM pH 7.8 buffer containing 1% SDS. Sorbic acid (solution 1%) was added as antibacterial agent.

Urine sample preparation

Each urine sample was treated as follows: an equivalent volume of bi-distilled water was added, followed by a centrifuge step at 1,500 g for 10 minutes. Aliquots of the supernatant were directly used for the measurements.

Toxin-like peptide identification

All samples have been analysed for the presence of proteins with potential toxic effect by using the cloud ion mobility mass spectrometry (CIMS) coupled with surface activated chemical ionization-Electrospray-NIST (SANIST) Bayesian model database search (SANIST-CIMS) as described in^{1,2}. The basics of this low voltage technology (no-discharge Atmospheric Pressure Chemical Ionization, APCI) has been adopted and used by the U.S. Food and Drug Administration³⁻⁵. The complete 'UniprotKB set of manually reviewed venom proteins and toxins'⁶

(mixed with a subset of not venom proteins and toxins from UniprotKB in order to give a statistical significance to the results) was used as reference protein dataset.

SANIST technology (surface-activated chemical ionization-electrospray-NIST) was used to obtain and compare the proteomic profiles. An Ultimate 3000 UPLC LC (by ThermoFisher) was used to achieve separation of analytes for each sample prior to MS analysis. A reversed phase C-18 LC column (50 × 2.1 mm; particle size, 5 µm; pore size, 100 Å, by Phenomenex, USA) was used. The eluent flow was 0.25 mL/min and the injection volume was 15 µL. The mobile phases were:

- A) 0.2% (v/v) formic acid (HCOOH)
- B) acetonitrile (CH₃CN).

The elution gradient was: 2% (v/v) of B between 0 and 2 min; 2 to 30% between 2 and 7 min; 30 to 80% between 7 and 9 min; 80% between 9 and 12 min; 80-2% between 12 and 12.1 min. The column was rebalanced with 2% of B between 12.1 and 17 min.

Samples were analysed with an HCT ion trap mass spectrometer (Breme, Germany) coupled to a surface activated chemical ionization (SACI) / ESI source and operated in positive and negative ion mode. Full scan spectra were acquired in the 40-3,500 m/z range for non-targeted metabolomics/proteomics analyses to detect analytes. The same m/z range was used for both discovery studies and selective biomarker studies in order to standardize the instrument response across the SANIST study, primarily in terms of scan rate.

The ion source parameters were:

- ESI capillary voltage: 1500 V
- SACI surface voltage: 47 V
- Desiccant gas: 2 L / min
- Nebulizer gas: 80 psi
- Temperature: 40 °C.

Mass spectrometry on samples was performed with collision-induced dissociation using helium as the collision gas. The ion trap was applied to isolate and fragment the precursor ions (windows of isolation, ± 0.3 m/z; collision energy, 30% of its maximum value, which was 5V peak to peak), and the Orbitrap mass analyser was used to obtain fragments with an extremely accurate m/z ratio (resolution 15,000; m/z error <10 ppm).

Similarity searches

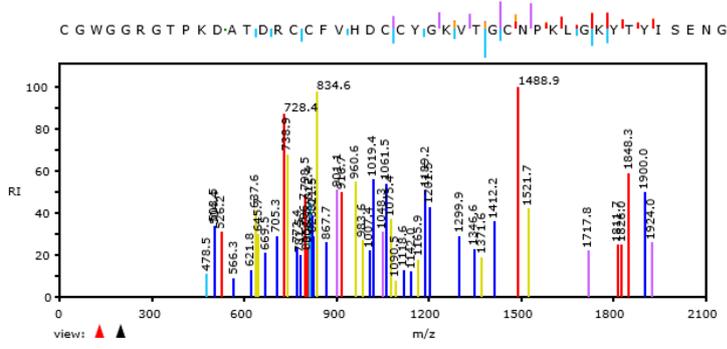
At the time of writing, TBLASTN⁷ was run at the National Center for Biotechnology Information (NCBI) website⁸ with default options and parameters, with the exception of the following ones: max target sequences = 1,000; expect threshold = 100; word size = 3; gap cost existence = 9; gap cost extension = 1; filter of low complexity regions = No. Searches have been performed versus: Nucleotide collection (nr/nt); Reference RNA sequences (refseq_rna); RefSeq Genome Database (refseq_genomes); Whole-genome shotgun contigs (wgs) from metagenomic experiments; Sequence Read Archive (SRA) sequences from metagenomic experiments; Transcriptome Shotgun

Assembly (TSA); Patent sequences(pat); Human RefSeqGene sequences(RefSeq_Gene); Betacoronavirus Genbank sequence dataset. The information reported in Table 1 has been retrieved from the UniprotKB⁹ database and from the NCBI Taxonomy database¹⁰.

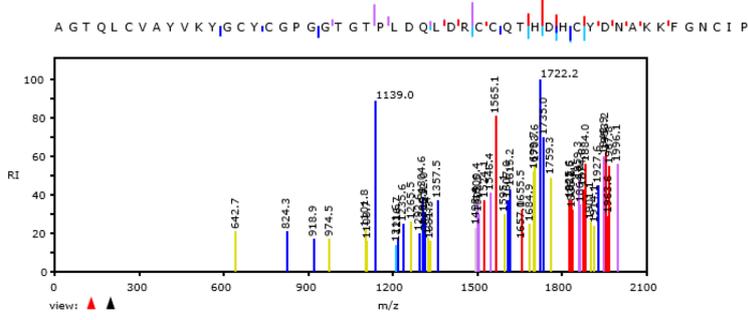
References

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5. Turnipseed SB, Andersen WC, Karbiwnyk CM, Roybal JE, Miller KE. No-discharge atmospheric pressure chemical ionization: evaluation and application to the analysis of animal drug residues in complex matrices. *Rapid Commun Mass Spectrom* 2006; 20: 1231–9.
6. UniprotKB. Animal toxin annotation project. <https://www.uniprot.org/program/Toxins> (accessed Oct 4, 2020).
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8. <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
9. <https://www.uniprot.org/>
10. <https://www.ncbi.nlm.nih.gov/taxonomy>

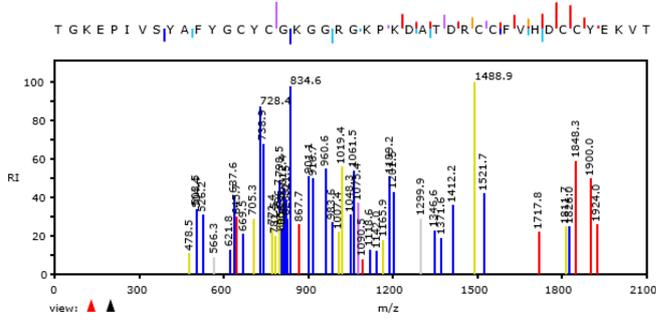
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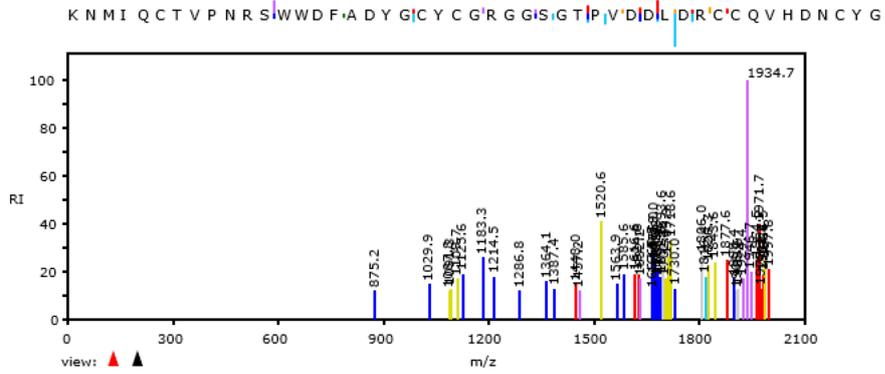
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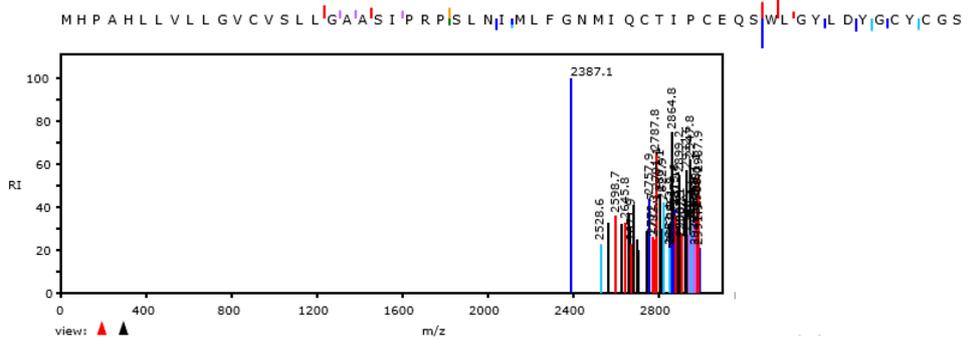
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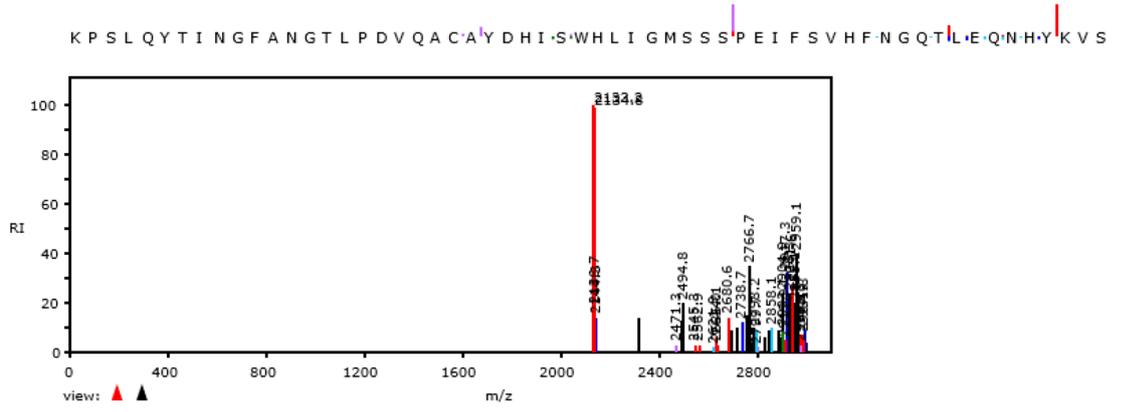
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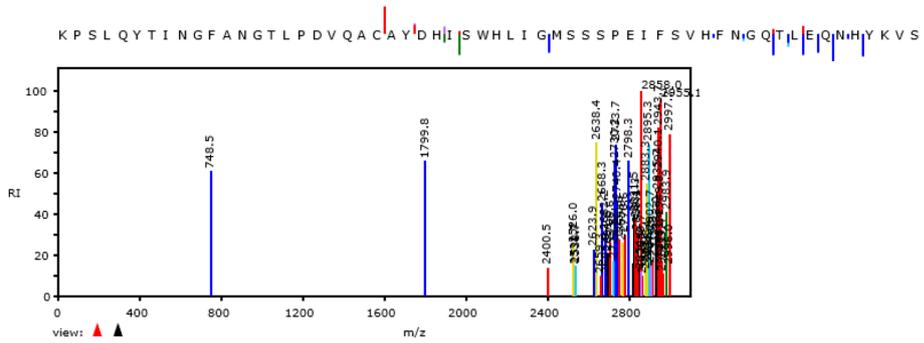
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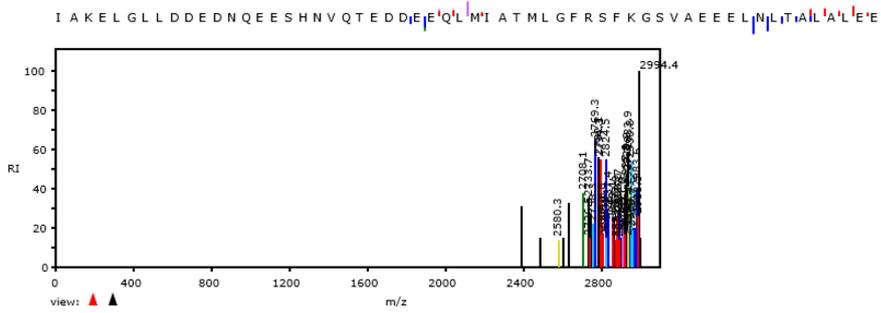
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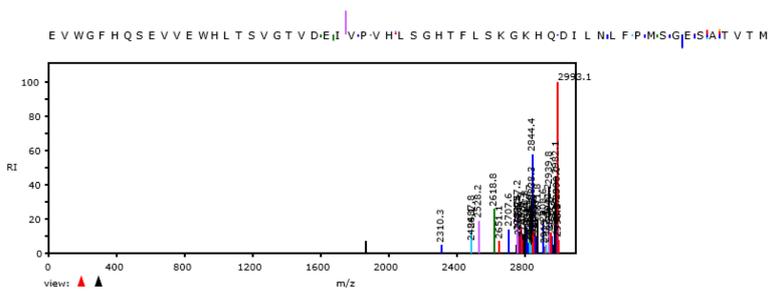
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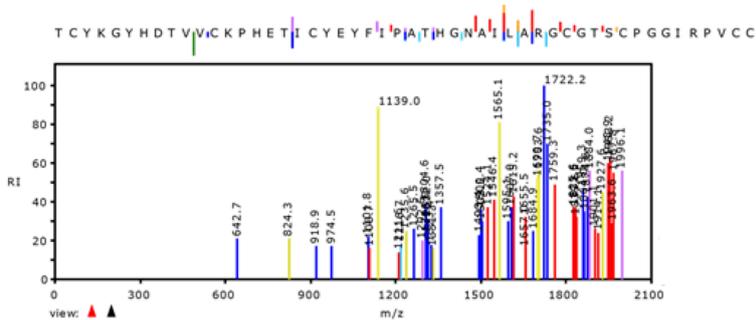
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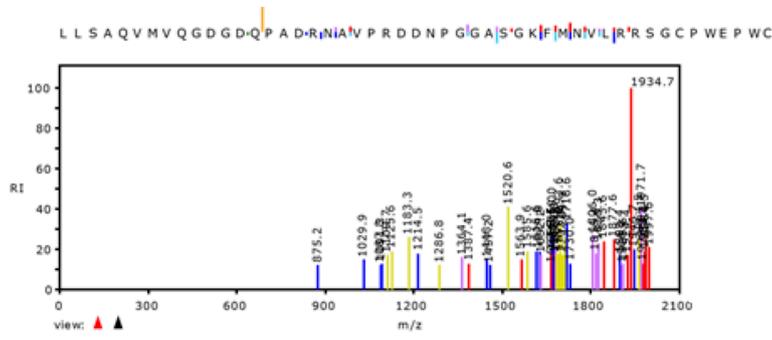
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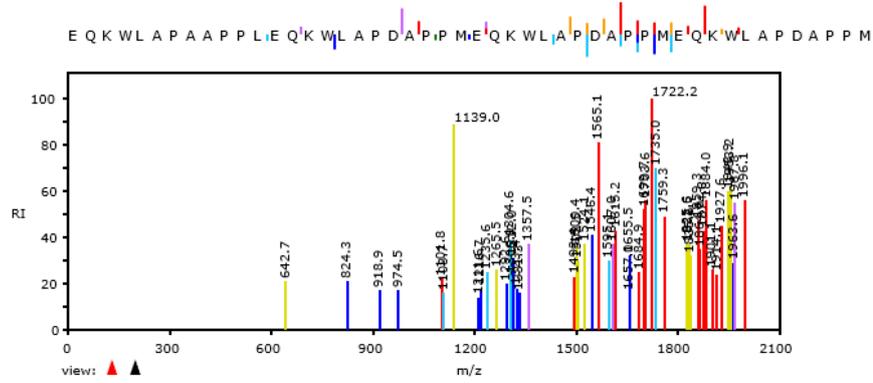
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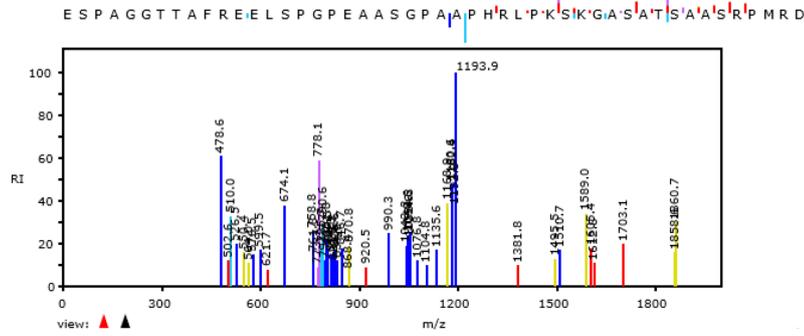
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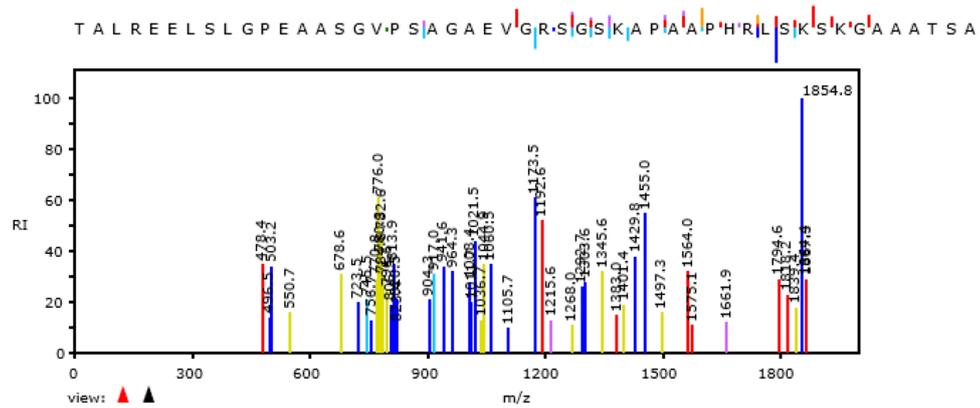
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